2012

The 'porin–cytochrome' model for microbe-to-mineral electron transfer

David Richardson
University of East Anglia, d.richardson@uea.ac.uk

Julea Butt
University of East Anglia

James K. Fredrickson
Pacific Northwest National Laboratory, jim.fredrickson@pnl.gov

John M. Zachara
Pacific Northwest National Laboratory, john.zachara@pnl.gov

Liang Shi
Pacific Northwest National Laboratory, liang.shi@pnnl.gov

See next page for additional authors

Molecular Microbiology (2012) 85(2), 201–212 ■
doi:10.1111/j.1365-2958.2012.08088.x
First published online 30 May 2012

MicroReview

The 'porin–cytochrome' model for microbe-to-mineral electron transfer

David J. Richardson,[1]* Julea N. Butt,[1] Jim K. Fredrickson,[2] John M. Zachara,[2] Liang Shi,[2] Marcus J. Edwards,[1] Gaye White,[1] Nanakow Baiden,[1] Andrew J. Gates,[1] Sophie J. Marritt[1] and Thomas A. Clarke[1]

[1]*Centre for Molecular and Structural Biochemistry, School of Biological Sciences and School of Chemistry, University of East Anglia, Norwich NR4 7TJ, UK.*
[2]*Pacific Northwest National Laboratory, Richland, WA, USA.*

Summary

Many species of bacteria can couple anaerobic growth to the respiratory reduction of insoluble minerals containing Fe(III) or Mn(III/IV). It has been suggested that in *Shewanella* species electrons cross the outer membrane to extracellular substrates via 'porin–cytochrome' electron transport modules. The molecular structure of an outer-membrane extracellular-facing deca-haem terminus for such a module has recently been resolved. It is debated how, once outside the cells, electrons are transferred from outer-membrane cytochromes to insoluble electron sinks. This may occur directly or by assemblies of cytochromes, perhaps functioning as 'nanowires', or via electron shuttles. Here we review recent work in this field and explore whether it allows for unification of the electron transport mechanisms supporting extracellular mineral respiration in *Shewanella* that may extend into other genera of Gram-negative bacteria.

Introduction

Many bacteria can couple anaerobic growth to the respiratory reduction of Fe(III) or Mn(III/IV) contained in oxide and phyllosilicate minerals. These solid substrates are abundant electron sinks for life on earth, but they are insoluble in water at neutral pH and consequently cannot enter the bacterial cells. So, to exploit these electron sinks, specific respiratory electron transfer mechanisms must overcome the physical limitations associated with electron transfer to extracellular terminal electron acceptors. Since there is a high natural abundance of Fe(III) in minerals, the substrate provides a massive electron sink for life in the planet's redox transition (or interfacial) zones. However, until very recently we have not understood how bacteria deliver electrons across their membranes to these insoluble minerals, with a number of different mechanisms being championed. Recent biochemical and structural work on proteins or homologues of proteins that are essential for this process in the *Shewanella* genus of metal-respiring bacteria is beginning to change this situation. We review this progress here and explore whether it allows for unification of the electron transport mechanisms supporting extracellular mineral respiration in *Shewanella*. We then explore whether such a unifying model can extend outside of the *Shewanella* genus into other genera of Gram-negative bacteria.

Energy-conserving electron transfer across two membranes

In the process of oxidative phosphorylation, ATP synthesis in Gram-negative bacteria relies on a proton-motive force across the inner (cytoplasmic) membrane, driven by energy from respiratory electron transport. During catabolism, electrons can reduce the inner-membrane quinone (Q) pool via a number of Q-reductases, including nicotinamide adenine dinucleotide (hydride NADH) dehydrogenase, formate dehydrogenase (Fdh; Fig. 1) and hydrogenase. The thermodynamic free-energy gap (ΔE 300–400 mV) in this process can generate a proton-motive force of ~ 200 mV across the membrane (Fig. 1). For turnover of the quinone reductases to be sustained, the reduced quinol (QH_2) pool must be continually re-oxidized. In the case of extensively studied soluble electron acceptors, such as O_2 and nitrate, the cognate QH_2 dehydrogenases are associated with the inner membrane and the active sites can be readily accessed by these water-soluble electron acceptors. Recycling the QH_2 pool is,

Accepted 1 May, 2012. *For correspondence. E-mail d.richardson@uea.ac.uk; Tel. (+44) 1603 593250; Fax (+44) 1603 592250.

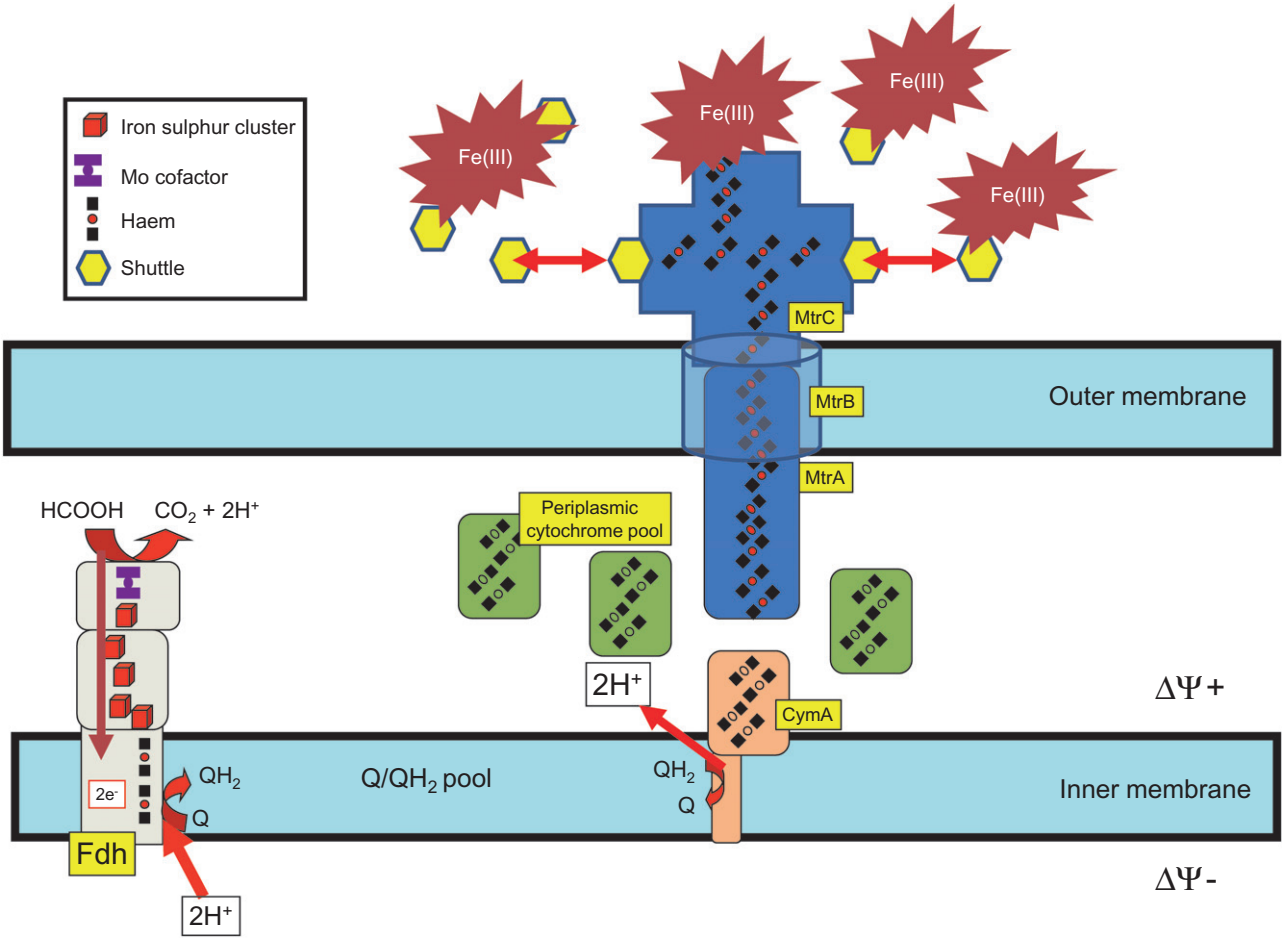

Fig. 1. A scheme for electron transfer from the inner cytoplasmic membrane to the extracellular environment in *Shewanella oneidensis*. The scheme is illustrated with formate as electron donor. The formate dehydrogenase (Fdh)-CymA redox loop couples the net movement of two positive charges from the membrane potential-negative (ΔΨ–) to the membrane potential-positive (ΔΨ+) side of the membrane per two electrons transferred in one Q/QH_2 cycle.

however, a challenge if the electron acceptor cannot enter the cell, as exemplified by Fe(III) and Mn(III/IV) solid phases. In this case the electrons must leave the QH_2 pool in the inner membrane, travel across the periplasm and the outer membrane to reach the microbe–mineral interface where a mechanism must exist to accomplish interfacial electron transfer with sites on the mineral surface. This process is explored in the sections that follow.

The Shewanella *'porin–cytochrome' model for electron transfer across the outer membrane*

It is widely accepted that *c*-type haems can play a major role in electron transfer to metal ions. These electron-carrying cofactors comprise of tetrapyrrole rings that co-ordinate a central Fe ion via the four pyrrole nitrogens. This 'haem' can carry one electron through the reduction of Fe(III) to Fe(II) and is covalently attached to the polypeptide chain via two cysteine residues in a CXXCH motif on the polypeptide chain. This motif makes recognition of *c*-type cytochromes in a proteome translated from a genome sequence relatively straightforward and many *Shewanella* species are predicted to have greater than 40 such cytochromes, a number of which bind more than one haem cofactor and are thus called multi-haem cytochromes (Heidelberg *et al.*, 2002).

In many species of Gram-negative bacteria, an inner-membrane QH_2 dehydrogenase (NapC/NrfH family) that binds four haems serves to recycle the QH_2 pool in the inner membrane. Mutation of the gene *cymA* encoding a NapC homologue in *Shewanella* species results in a deficiency in mineral metal respiration (Myers and Myers, 2000). CymA can be modelled on the recently solved NrfH structure, which reveals a tetra-haem chain that can move electrons approximately 4 nm (i.e. ~ 30–40% of the width of the periplasm) (Fig. 1) (Zargar and Saltikov, 2009). Thus, CymA could deliver electrons from the inner-membrane QH_2 into the periplasm and so recycle the Q

pool (Fig. 1). A number of multi-haem cytochromes are predicted to be located in the periplasm of *Shewanella* species, including the small tetra-haem cytochrome (Stc) and the tetra-haem domain of the fumarate reductase flavocytochrome c_3 (Fcc_3), which can potentially receive electrons from CymA (Ross *et al*., 2007; Schuetz *et al*., 2009). However, recent results suggest that none of these play a critical role in extracellular Fe(III) oxide reduction by *Shewanella oneidensis* MR-1 (Schuetz *et al*., 2009; Coursolle *et al*., 2010). It cannot be excluded that one or more of these can participate in shuttling electrons from the inner-membrane CymA across the periplasm to the inner face of the outer membrane, but there is most likely functional overlap among the pool of periplasmic multi-haem cytochromes in *Shewanella*.

In *Shewanella* species the challenge of moving periplasmic electrons from the inner to outer face of the cell membrane is solved by a protein complex that comprises a 40 kDa deca-haem cytochrome (MtrA) and a trans-outer-membrane β-barrel protein (MtrB). Models of MtrA suggest that it contains two ~ 150 amino acid modules with homology to the *Escherichia coli* NrfB protein. NrfB is a 4 nm electron-transferring penta-haem 'wire' and so two end-to-end NrfB molecules would yield a protein of around 8 nm in length (Clarke *et al*., 2007; 2008). This is broadly consistent with a recent low-resolution structure of recombinant MtrA derived from small-angle X-ray scattering (SAXS) that suggested a length of around 10 nm (Firer-Sherwood *et al*., 2011). A role for MtrA in electron transfer across the outer membrane was initially considered unlikely since *in silico* analysis predicts it to be a soluble periplasmic protein. However, native MtrA actually associates strongly with the periplasmic face of the outer-membrane fraction in *S. oneidensis* and it has recently been established that this is because it forms a tight complex with the integral outer-membrane protein MtrB (Ross *et al*., 2007; Hartshorne *et al*., 2009).

Modelling of MtrB suggests that it comprises a 28 strand trans-membrane barrel that could form a pore some 3–4 nm in diameter (Hartshorne *et al*., 2009). Genetic studies suggest that the *mtrB* gene is critical to the Fe(III)-respiring process. However, MtrB's role in electron transfer across the outer membrane has been unclear since its amino acid sequence does not have an obvious redox cofactor-binding motif. As MtrB is predicted to be a large porin-type protein, one solution for moving electrons across the outer membrane is to embed the deca-haem MtrA within the MtrB barrel (Fig. 1) (Ross *et al*., 2007; Hartshorne *et al*., 2009). In this way MtrA provides the outer membrane-spanning electron wire, which is insulated by an MtrB sheath – this is the 'porin–cytochrome' electron transfer module. In principle, such an MtrAB complex could span the outer membrane and also extend into the periplasm where it could contact other periplasmic electron transfer proteins. There is also a possibility that MtrA could protrude sufficiently into the periplasm to interact directly with inner-membrane CymA, thus providing the link of the outer membrane to the proton-motive inner-membrane electron transport system (Fig. 1). This would depend on how deeply MtrA is embedded into the interior of MtrB β-barrel. The recent analysis of recombinant MtrA by SAXS suggested that MtrA might be too wide to embed deeply into the barrel (Firer-Sherwood *et al*., 2011). However, it should be recognized that as yet there is no structure for MtrB and so the dimensions of the internal cavity cannot be known with any certainty.

What is the mechanism for microbe-to-mineral electron transfer?

The model presented thus far can explain how electrons are conducted from inside the cell to the extracellular environment via outer-membrane porin–cytochrome electron transport complexes. However, it has been the subject of a debate whether, once outside the cells, electrons are transferred: (i) directly from outer-membrane cytochromes to insoluble electron sinks, (ii) by assemblies of cytochromes, perhaps associated with extracellular appendages, sometimes referred to as nanowires or (iii) via electron shuttles (Fig. 1). There is an emerging consensus that a family of extracellular deca-haem cytochromes plays an important role in all three models. This is the MtrC family, which in *Shewanella* includes the homologues OmcA and MtrF. The extracellular location of these deca-haem cytochromes has been established by a range of biophysical studies and through the demonstration that they are exported by the type II secretion system (DiChristina *et al*., 2002; Donald *et al*., 2008; Shi *et al*., 2008; Lower *et al*., 2009; Reardon *et al*., 2010). All three proteins have been extensively characterized spectropotentiometrically and it has been shown that the 10 haems within each protein oxidize and reduce across a very broad potential window between ~ +100 and ~ –500 mV (pH 7 versus the standard hydrogen electrode) and readily exchange electrons at high rates with graphite and, at slower rates, hematite electrodes in the absence of redox mediating electron shuttles (Hartshorne *et al*., 2007; Eggleston *et al*., 2008; Firer-Sherwood *et al*., 2008). MtrC copurifies with MtrAB from *S. oneidensis*, and the functionality of the MtrABC complex in trans-membrane electron transfer was demonstrated in sealed proteoliposomes (Hartshorne *et al*., 2009). Further support for a role for MtrCAB in trans-membrane electron transfer comes from expression of *mtrCAB* in *E. coli* that confers a capacity for hematite reduction on the recipient organism (Jensen *et al*., 2010). It should be noted though that the iron-reducing activity reported was very low com-

pared to *S. oneidensis* and so the question of whether MtrCAB on its own is sufficient to confer high rates on mineral iron respiration remains.

Significant insights into the mechanism of microbe-to-mineral electron transfer were recently provided by the resolution of the structure of MtrF, a member of the MtrC family, that may be functionally associated with an MtrDE porin–cytochrome complex (homologous to MtrAB). The *mtrDEF* genes are most highly expressed in aggregated cell cultures of *S. oneidensis*, although as yet a clear phenotype of an MtrF mutant has yet to be established (McLean *et al*., 2008; Clarke *et al*., 2011). MtrF consists of four domains (Fig. 2A). Five tightly packed haems are covalently attached to domains II and IV, while domains I and III each contain seven anti-parallel β-strands folded together through an extended Greek key topology that results in a split β-barrel structure. The overall haem organization is a 'staggered cross', in which a staggered 65 Å octa-haem chain transects the length of the protein and is crossed at the middle by a 45 Å tetra-haem chain that connects the two split β-barrel domains. Each haem is within 7 Å of its nearest neighbour(s), which will allow for rapid electron transfer through the protein (Fig. 2B). The overall arrangement suggests that MtrF is optimized for trifurcated electron transfer across both the cytochrome domains and into the flanking β-sheet domains via the quadri-directional staggered haem 'cross–roads' junction. A *c*-type haem has two propionate side chains and, thus, a deca-haem cytochrome has 20 negatively charged propionates. MtrF binds 18 calcium ions that may contribute to charge compensation for these propionates (Clarke *et al*., 2011) and so it is perhaps notable that in *Shewanella* sp. HRCR-1, the extracellular cytochromes have been shown to be embedded in extracellular polymers (EPS) that are rich in calcium ions (Cao *et al*., 2011).

MtrF shares 30% identity and 46% similarity with MtrC and the sequence of alternating non-haem domains and penta-haem domains is conserved. Thus, the crystal structure of MtrF serves as the prototypical structure for the 'MtrC family'. From a physiological view point multi-haem *c*-type cytochromes can be grouped broadly into two functional classes. The first serves as periplasmic electron wires, such as Stc and penta-haem NrfB (Clarke *et al*., 2007), and tends to have a low protein-to-haem ratio (~ 3 kDa protein/haem). The second has a catalytic function, such as the penta-haem nitrite reductase or octa-haem hydroxylamine oxidoreductase and tetrathionate reductase. These exhibit a high protein-to-haem ratio (~ 10 kDa protein/haem) because of the requirements for the protein to form a scaffold to hold multiple haems and contain sufficient additional polypeptide to construct an active site with substrate/product channels (Clarke *et al*., 2008). The crystal structure of MtrF reveals a hybrid of these two classes; the penta-haem domains I and III have a low protein-to-haem ratio of ~ 3 kDa protein/haem comparable to that of the electron shuttling multi-haem cytochromes, but inclusion of the domains formed by the Greek key split β-barrel domains gives an overall protein-to-haem ratio for MtrF of 7 kDa protein/haem that is closer to that of the catalytic multi-haem cytochromes. In the context of mineral respiration this suggests multiple activities for this protein that may allow formulation of a unifying model for extracellular electron transfer in *Shewanella* that explains experimental observations of direct 'microbe-to-mineral' electron transfer, indirect 'microbe-to-shuttle-to-mineral' electron transfer and electron transfer via nanowires.

Cytochrome-mediated 'microbe-to-mineral' electron transfer

The question of whether the outer membrane-associated deca-haem cytochromes, MtrC, MtrF and OmcA, can bind to, and directly reduce, metal oxides such as reactive ferrihydrite, or the more stable goethite (FeOOH) or hematite (Fe_2O_3) has been controversial and has thus received a great deal of experimental attention. It is a challenging question to address. First, these oxides range significantly in their free energy, crystallinity and hence solubility, reduction potential and overall reducibility. Their reduction potentials range from a high of +61 mV for ferrihydrite (pH 7.0) to a low of –230 mV for hematite (pH 7) (Fig. 3). Fe(III) oxide–cytochrome interaction studies are necessarily performed *in vitro*, using nano- and microparticulate aqueous suspensions of laboratory synthesized oxides of controlled properties. However, the partially hydrophobic character of the cytochromes requiring a solubilizing agent to maintain them in solution and the tendency for the oxides to aggregate pose serious challenges for artefact-free kinetic studies. Consequently, defensible electron transfer rates have not been published. Additionally, interfacial orientation and approach distance are important variables influencing interfacial cytochrome electron transfer rates (Kerisit *et al*., 2007), and it is unclear whether optimal interfacial configurations or associations assemble abiotically under *in vitro* conditions. Hematite is the only one of these common Fe(III) oxides that can be synthesized or obtained in extended single crystal form enabling direct microscopic, spectroscopic and electrochemical studies of interfacial structure, orientation and electron transfer rate, and important insights have resulted (e.g. Lower *et al*., 2007; 2009; Eggleston *et al*., 2008; Meitl *et al*., 2009; Johs *et al*., 2010). The thermodynamic properties of hematite, however, make it a relatively weak oxidant with a relatively narrow range of cytochrome redox reactivity. Ferrihydrite displays a much larger redox range and is more environmentally relevant, but it exists as 5–8 nm nanoparticles that profusely aggregate and readily transform to other

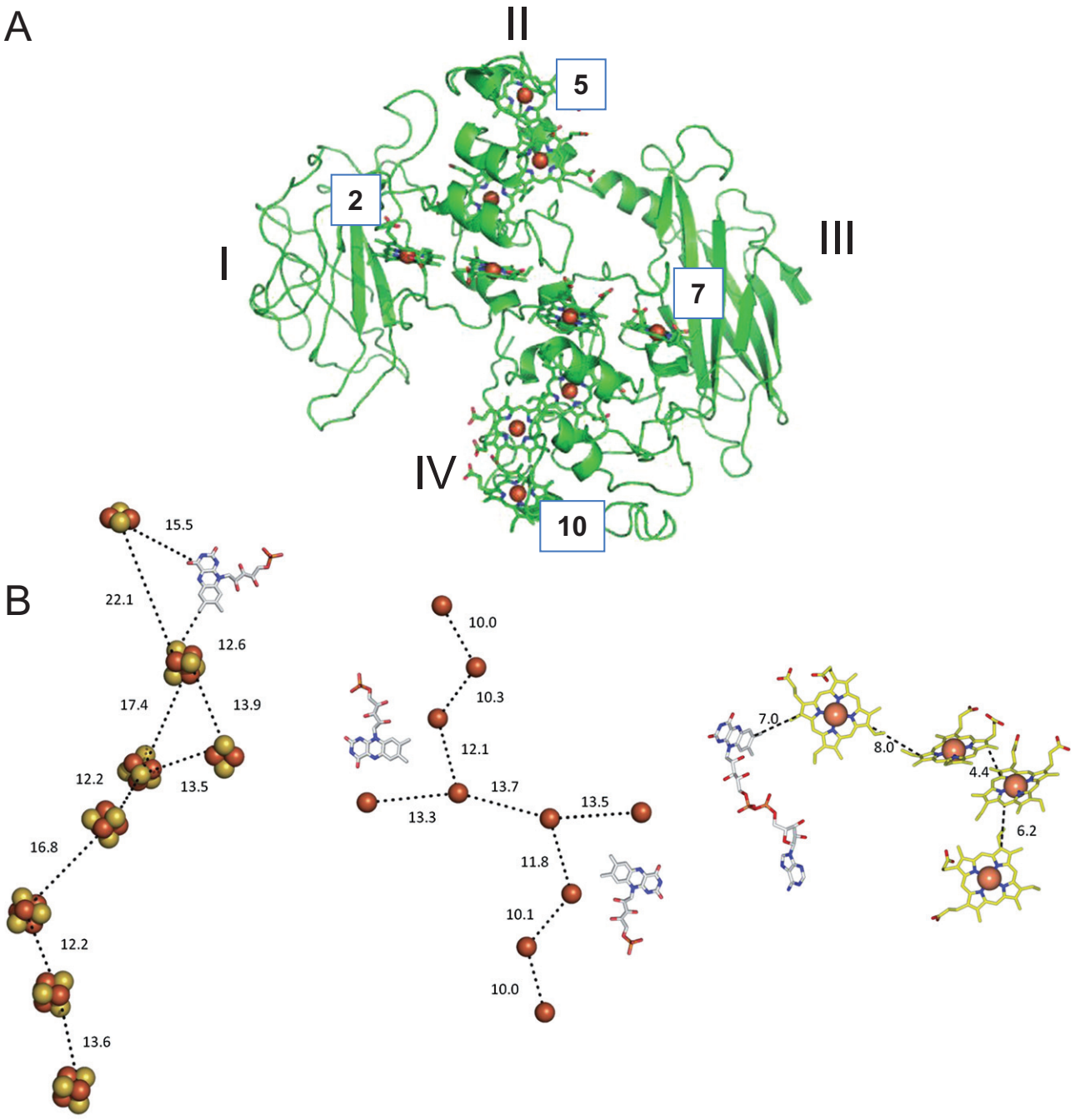

Fig. 2. The organization of multi-redox centred electron wires.
A. The molecular structure of *S. oneidensis* MtrF (adapted from Clarke *et al*., 2011).
B. Left, the iron sulphur cluster electron transfer wire of *Thermus thermophilus* NADH dehydrogenase (adapted from Roessler *et al*., 2009). Centre, the position of the iron atoms in multi-haem electron transfer wire from *S. oneidensis* MtrF (adapted from Clarke *et al*., 2011). The two flavin molecules are placed in hypothetical positions to illustrate a possible route of electron transfer. Right, the tetra-haem/flavin adenine dinucleotide electron transport chain of FccA (adapted from Taylor *et al*., 1999).

Fe(III) oxide phases subsequent to interfacial electron transfer. These factors complicate the quantification of interfacial contact area and site reaction stoichiometries that are requisite for kinetic analysis.

A combination of measurements with fluorescence correlation spectroscopy, optical waveguide lightmode spectroscopy and protein film voltammetry (PFV) have shown that OmcA and MtrC can bind to hematite in an ionic-

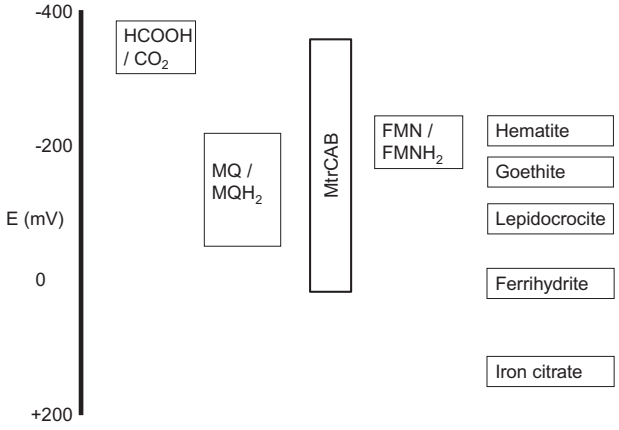

Fig. 3. The midpoint redox potentials of selected electron transfer proteins and substrates involved in extracellular Fe(III) respiration. Potentials are given for pH 7. The boxes represent approximate span of the potentials from 90% reduced to 90% oxidized.

strength and pH-dependent manner (Xiong *et al*., 2006; Lower *et al*., 2007; 2008; 2009; Eggleston *et al*., 2008). PFV measurements have also shown that MtrC, OmcA and MtrF can transfer electrons directly to graphite electrodes with interfacial electron transfer rates that lie in range of ~ 100 to 300 per second (Hartshorne *et al*., 2007; 2009; Firer-Sherwood *et al*., 2008; Clarke *et al*., 2011). Inspection of the MtrF structure suggests electron input/egress sites via haems 5 and 10, which are the most solvent exposed haems (Fig. 2A). These haems are positioned so that the edges of the porphyrin rings are exposed to the solvent, a configuration that has been shown to be optimal for electron transfer to insoluble minerals (Smith *et al*., 2006).

Using phage display technology a peptide with hematite-binding motif has been identified in MtrC and OmcA that has a conserved sequence of Ser/Thr-hydrophobic/aromatic-Ser/Thr-Pro-Ser/Thr (Lower *et al*., 2007; 2008). Molecular dynamic simulations with the peptide Ser-Pro-Ser indicated that hydrogen bonding occurs between two serine amino acids and the hydroxylated hematite surface and that the proline induces a structure-binding motif by limiting the peptide flexibility. However, this sequence cannot be identified on the MtrF structure and so the importance of this peptide is unclear at present. The shape of OmcA in solution has been imaged using SAXS (Johs *et al*., 2010). It has similar dimensions ($34 \times 90 \times 65$ Å) to that of MtrF ($30 \times 85 \times 70$ Å). Neutron reflectometry showed that OmcA forms a well-defined monomolecular layer on hematite surfaces, where it assumed an orientation that maximized its contact area with the mineral surface (Johs *et al*., 2010). The exact relationship of the conformation of OmcA on a surface and MtrF in a mineral-free state will require further study, perhaps by undertaking atomic force microscopy studies with MtrF on hematite surfaces and trying map the known structure onto the images obtained.

Extracellular inter-cytochrome electron transfer and nanowires

The deca-haem cytochromes are peripheral membrane proteins, not integral membrane proteins like MtrB, and are readily washed from the cell surface. Protein purification and *in vivo* cross-linking experiments have demonstrated that, in addition to forming a complex with MtrAB, MtrC can also associate with OmcA (Shi *et al*., 2006; Ross *et al*., 2007; Zhang *et al*., 2008; 2009). For example, a heterotrimeric complex between OmcA and MtrC (2:1) has been characterized *in vitro* (Shi *et al*., 2006; Ross *et al*., 2007; Zhang *et al*., 2008). This raises the possibility that outer-membrane deca-haem cytochromes can associate to form electron transport chains that extend beyond the cell surface. From the MtrF structure it is evident that inter-cytochrome electron transfer could occur if the proteins interact via domains II and IV that brings haems 5 and 10 of the partner proteins close enough for rapid interfacial electron exchange (Fig. 4). If this was the case, a chain of ~ 30 nm could form from the $(OmcA)_2$: MtrC heterotrimer, and much longer chains could be envisaged in a stable structured biofilm, perhaps in an extracellular polysaccharide (EPS)–lipid–protein matrix. The possibility that OmcA might be particularly important in inter-cytochrome electron transfer through extracellular cytochrome assemblies is supported by examination of the distribution of MtrC and OmcA in EPS. Two forms of EPS can be isolated from *Shewanella* sp. HRCR-1 that, like *S. oneidensis*, also has genes encoding the MtrCAB and OmcA systems. The EPS forms are referred as 'tight' and 'loose' and it is suggested that they are close to and more distant from the outer membrane respectively. MtrC was predominantly distributed into the tight EPS fraction, while OmcA was equally distributed between the tight and loose fractions (Cao *et al*., 2011). This is suggestive of OmcA playing a role in distributing electrons away from the MtrCAB complexes through the extracellular EPS matrix.

Pilus-like structures, or nanowires, have also been proposed to play roles in metal reduction and current production in microbial fuel cells, although the biological relevance of these remains unclear. Preliminary experiments have shown nanofilaments of *Shewanella* to be conductive, although the mechanism and extent of their conductivity have not been determined (Gorby *et al*., 2006. There is also controversy over whether imaged nanofilament morphologies have resulted from dehydration of EPS during sample preparation and analysis (Dohnalkova *et al*., 2011). Early measurements of electrical conductance by nanowires were limited to measurements across the thickness of the 'wire', but recently electrical conduc-

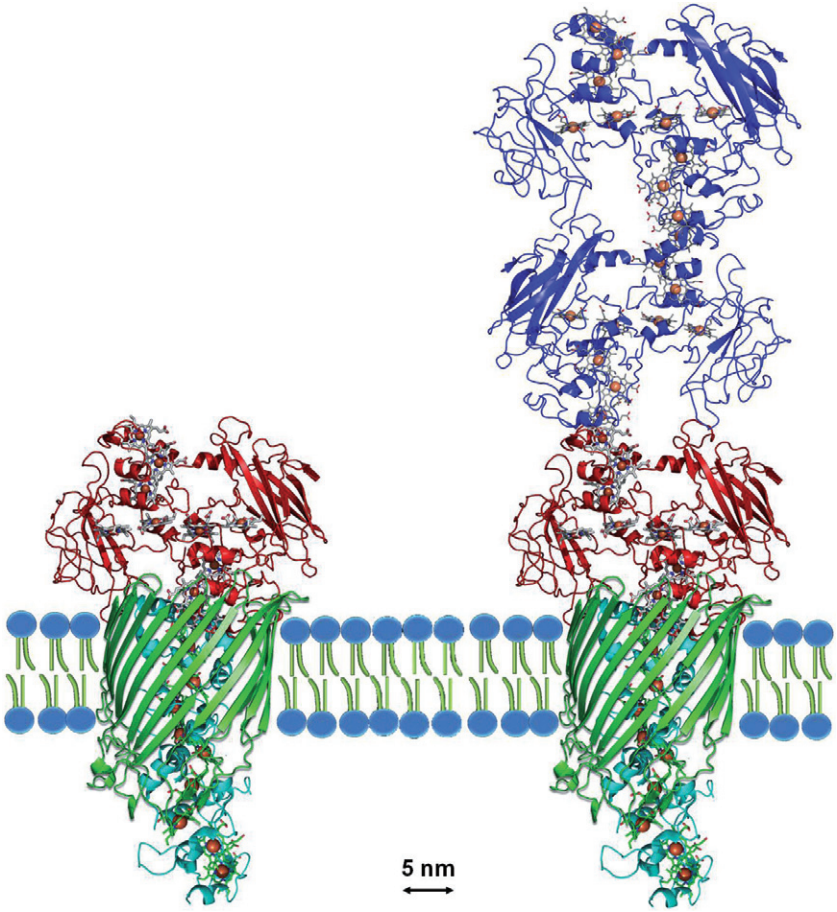

Fig. 4. A cartoon showing a possible molecular configuration for an MtrCAB-type porin–cytochrome complex and extracellular MtrC–OmcA cytochrome chains. The structures of the deca-haem cytochromes MtrC (red) and OmcA (blue) are based on that of MtrF (Clarke *et al.*, 2011). The structure of MtrA (lilac) is based on that of two penta-haem NrfB monomers fused end to end (Clarke *et al.*, 2007). The degree to which the multi-haem cytochromes embed into the porin sheath is not currently known and this cartoon is therefore purely illustrative of one possible arrangement.

tivity along the length of the wire has been demonstrated (El-Naggar *et al.*, 2010). Pili are not essential for extracellular electron transfer in *Shewanella* (Bouhenni *et al.*, 2010), but this does not exclude a role in formation of nanofilament assemblies that contribute to extracellular electron transfer. In *Shewanella* the appendages synthesized by an *mtrC*/*omcA* double mutant are not electrically conductive (Gorby *et al.*, 2006). There could be two explanations for this: (i) the MtrCAB–OmcA complex is required to move electrons out of the cell to keep the wire charged and/or (ii) MtrC/OmcA are required for conductance along the length of the wire. At present it is not possible to distinguish these two possibilities.

Cytochrome-mediated 'microbe-to-shuttle-to-mineral' electron transfer

Firm evidence for the importance of electron shuttles in extracellular electron transfer came from experiments in which mineral Fe(III) oxide deposited in porous glass beads could still be reduced by *Shewanella* (Lies *et al.*, 2005). Additional experimentation with this model system revealed that *Shewanella* could reduce and extract ferrihydrite from physically inaccessible intra-grain regions and precipitate Fe(II) mineral forms on the grain surface proximate to their areas of colonization (Peretyazhko *et al.*, 2010). Further corroboration came from studies on the molecular mechanism by which *S. oneidensis* adheres to and respires Fe(III) oxides using surface-associated serine proteases that function as bacterial adhesins. A mutant in serine protease SO3800 was impaired in its ability to adhere to Fe(III) oxides but retained wild-type Fe(III) respiratory capability, indicating that *S. oneidensis* can respire insoluble Fe(III) oxides at a distance, in addition to via direct contact (Burns *et al.*, 2010). A recent study using nanoelectrodes monitored by *in situ* optical imaging measured currents that were not correlated with the cell number on the electrodes, suggesting that electron transfer occurs predominantly by a shuttle-mediated mechanism in this model system (Jiang *et al.*, 2010).

Shewanella oneidensis MR-1 cells have been shown to secrete water-soluble riboflavin and flavin mononucleotide (FMN) and these appear to facilitate extracellular electron transfer to Fe(III) oxides and electrodes (Marsili *et al.*, 2008; von Canstein *et al.*, 2008; Baron *et al.*, 2009; Coursolle *et al.*, 2010). The exact mechanism by which flavins reduce Fe(III) oxides remains undetermined, but flavin mediated Fe(III) reduction is compromised in MtrAB and

MtrC/OmcA mutants, suggesting a role for the porin–cytochrome complex and the extracellular deca-haem cytochromes in electron exchange with the flavin. MtrC and MtrB are also required for reduction of the artificial extracellular electron shuttle, anthraquinone-2,6-disulfonate (AQDS), which is an analogue of redox-active components found in humics (Lies *et al*., 2005). The broad redox potential operating ranges of the extracellular cytochromes makes it thermodynamically possible to transfer electrons directly to both flavins and AQDS, which have midpoint potentials in the range of –100 to –200 mV (pH 7 versus SHE) (Fig. 3). It should be noted that most Fe(III) reduction in soils and sediments occurs with distributed nanoparticulate oxides that do not present an oriented or well-defined surface for microbes to adsorb to. The advantage of diffusible reductants such as flavins is to bypass the physical restriction of this small oxide particle size as flavins are excellent and rapid reductants that can function without complexation.

The structure of MtrF gives tantalizing clues as to how direct and flavin-mediated electron transfer to minerals could operate together to sustain electron transfer. The Greek key β-barrel motif of domains I and III is a common fold among FMN-binding domains, where the barrel interior contains hydrophobic residues and the barrel surface contains predominantly charged residues. This is therefore consistent with the electron shuttling model whereby a flavin could bind transiently to these canonical flavin-binding domains. Two haem termini (haems 2 and 7) are within 14 Å of the centre of the nearest β-barrel domain, which would allow electrons to be rapidly transferred into flavin shuttles bound to the flanking domains (Fig. 2). It is notable that, unlike Fe(III) reduction, flavin reduction is a two-electron reaction and domains I and III may serve to stabilize a semireduced state until the second electron passes to bound semiquinone or the flavin may bind close to two haems (e.g. Fig. 2) to allow for a concerted two-electron transfer. This will require further investigation, but it is notable that precedents for multi-haem chains reducing bound flavin can be identified in another structurally defined group of *Shewanella* multi-haem cytochromes, the Fcc_3 tetra-haem flavocytochrome *c* fumarate reductases. Here electron input to the flavin takes place in rapid sequential single-electron transfer steps from the terminal haem IV of the chain that is in turn very rapidly re-reduced by the upstream haem III, minimizing the lifetime of the potentially reactive semiquinone (Fig. 2B) (Taylor *et al*., 1999; Pessanha *et al*., 2009).

The MtrF structure appears to explain the observations that the MtrC family of outer-membrane cytochromes is required for flavin reduction. The question then is how flexible is this site for reduction of other soluble molecules? For example, can it mediate binding and reduction of AQDS and soluble Fe(III) chelates? Resolution of this will require further studies, including site-directed mutagenesis of possible shuttle-binding residues. Electron shuttles could also function in cytochrome assemblies since the predicted inter-cytochrome electron transfer sites (haems 5 and 10, Fig. 2) from the MtrF structure are different from the more buried putative electron exchange sites with electron shuttles (haems 2 and 7). Flavins could consequently shuttle electrons from cytochrome assemblies associated with extracellular structures such as pili or EPS component scaffolds.

The relative importance of direct versus mediated electron transfer to mineral materials may change in different environments and, in light of the MtrF structure, it seems likely that the two processes can occur in tandem (Clarke *et al*., 2011). The thermodynamic domain in which free FMN operates is ~ –200 to –100 mV. This is consistent with an electron shuttling role since a shuttle needs to be sufficiently oxidizing to extract reductant from the multihaem cytochrome, but sufficiently reducing to enable it to pass on these electrons to an Fe(III) complex or solid (Fig. 3). Consistent with this model, FMN only partially oxidizes reduced MtrF, suggesting that electron egress via domains I and III (Fig. 2) represents low potential branches of the MtrF haem network with termini tuned for FMN reduction, while the terminus of the octa-haem chain spanning domains II and IV represents a higher potential branch more tuned to electron transfer to Fe(III) minerals (Clarke *et al*., 2011). A multi-mechanism electron transfer model is attractive for natural environments including marine and freshwater sediments, soils and subsurface materials where solid-phase electron acceptors exist as dispersed nano- and microcrystallites with complex surface morphologies and structures complicating electron transfer by direct contact. Moreover, these crystallites are often aggregated or reside in physical locations such as grain coatings or microfractures within lithic fragments with size dimensions that prevent direct contact with the cell envelope. A soluble, diffusible electron transfer agent or extended extracellular network of cytochromes, low molecular weight shuttles and EPS scaffold can bypass these impediments that challenge a cell surface-tethered protein.

Shewanella porin–cytochrome mediated electron transfer as a unifying model for outer-membrane electron transport?

The previous discussion has illustrated how different mechanisms of microbe-to-mineral electron transfer based on cytochromes, electron shuttles or nanowires may, in fact, be subgroups of a common mechanism that is ultimately dependent on electron transport across the outer membrane via porin–cytochrome complexes (Fig. 1). The respiratory flexibility of *Shewanella* species includes the ability to utilize dimethylsulfoxide (DMSO) as a respiratory

electron acceptor. In *E. coli* the active site of the DMSO reductase, which is a molybdoenzyme, is located in the periplasmic compartment. However, *Shewanella* are configured to respire extracellular forms of DMSO that may be abundant in oceans by localizing the Mo-containing catalytic subunit to the outside of the cell (Gralnick *et al*., 2006). The genes encoding the catalytic subunits in *Shewanella*, *dmsA* and *dmsB*, cluster with genes *dmsE* and *dmsF* which are predicted to encode homologues of MtrA and MtrB. Thus, it is likely that respiratory DMSO reduction uses a similar mechanism for moving electrons across the outer membrane as found in extracellular mineral respiration. Bioinformatic analysis also suggests that MtrABC homologues are present in some *Vibrio* species (three out of 30 species we have surveyed).

Alongside *Shewanella* the best studied iron-respiring genus is *Geobacter*, which appears to produce electrically conducting nanowires (Reguera *et al*., 2005; 2006), but in which there are no clear homologues of the MtrC/MtrF/OmcA family. This is intriguing since *Geobacter* species are not known to excrete flavins. Thus, there may be a correlation between the synthesis of the MtrC deca-haem family and secretion of flavins by bacteria. *Geobacter* does, however, secrete other extracellular cytochromes and one of these, the hexa-haem OmcS (Qian *et al*., 2010), has been reported to be associated with the pilus nanowires. Whether OmcS plays a role in current conduction along the length of the wire or simply as a contact point for mineral iron (III) reduction is not clear (Leang *et al*., 2010). A recent study has presented evidence for *Geobacter* pili being electrically conductive through an intrinsic metallic-like conductivity mechanism (Malvankar *et al*., 2011). There is though active debate in the literature between advocates of this mechanism and advocates of an electron super-exchange mechanism involving the redox cofactors of multi-haem cytochromes (Strycharz-Glaven *et al*., 2011; Malvankar *et al*., 2012; Strycharz-Glaven and Tender, 2012). This debate will be aided if molecular resolution of the conducting pili and the pili–cytochrome interactions can be resolved. However, in either mechanism of electrical conduction, the pili will need to be 'charged' by electrons generated from intracellular catabolism. It is therefore notable that there are homologues of *mtrA* in some *Geobacter* species that cluster with genes predicted to encode large β-barrels; for example, genes BK32R6 and BK32R7 from *Geobacter* sp. M21 share 52% and 68% similarity with *S. oneidensis* MtrB and MtrA respectively (Hartshorne *et al*., 2009). This raises the possibility that electron transfer through the outer-membrane porin–cytochrome complexes charges the pili. A porin that appears to be important for mineral iron respiration in *Geobacter sulfurreducens* is OmpJ (Afkar *et al*., 2005). Other than both being outer-membrane β-barrel proteins OmpJ and MtrB appear quite different, with OmpJ predicted to have 16 trans-membrane strands compared to the 28 strands of MtrB. However, an *ompJ* mutation in *G. sulfurreducens*, like an *mtrB* mutation in *S. oneidensis*, has a detrimental effect on mineral iron respiration and outer-membrane cytochrome assembly (Afkar *et al*., 2005). This is suggestive of a common function, and so perhaps an example of convergent evolution.

Homologues of MtrA and MtrB (PioA and PioB) are associated with Fe(II) oxidation in the phototroph *Rhodopseudomonas palustris* (Jiao and Newman, 2007) and *Sideroxydans lithotrophicus* ES-1 (MtoA and MtoB), which grows on $FeCO_3$ or FeS at oxic–anoxic interfaces at circumneutral pH (Liu *et al*., 2012). In both cases the bacterial-mediated Fe(II) oxidation occurs extracellularly and so electrons must be moving into, rather than out of, the cell, through the outer-membrane cytochrome–porin complex. Thus, the *S. oneidensis* MtrAB module appears to represent a widely used solution to electron transport across the outer membrane, with functional diversity probably being achieved by non-conserved regions that confer specificity for interactions with distinct extracellular proteins. Questions remain, however, on the structure and dynamic function of this module. For example, is the position of the MtrA component within MtrB modulated to allow MtrA to extend to different lengths into the periplasm or the extracellular space in different systems? Could this modulation be dynamic such that docking of MtrC or OmcA on to MtrB results in structural changes that allow MtrA to engage with extracellular cytochrome? These possibilities will require further experimentation to explore.

The basic model of an outer-membrane β-barrel sheath housing a redox protein partner could expand beyond MtrB and its homologues. Genome analyses reveal porin-type proteins of unknown function in iron-respiring bacteria that do not have Mtr-type cytochromes, but in which other water-soluble cytochromes or redox proteins and outer-membrane porins are implicated in electron transfer to the extracellular minerals. Thus, embedding redox proteins into outer-membrane β-barrels may provide a widespread mechanism for 'water-soluble' redox proteins that are predicted to be periplasmic, to gain access to the extracellular environment in a phylogenetically diverse range of bacteria. In this respect it is important to think outside of the 'haem box'. The structures of a number of redox chains associated with the inner membrane of bacteria that contain insulated 'wires' of iron sulphur clusters have emerged; for example, the NADH dehydrogenase where such a chain of one electron-transferring centres leads to a two-electron reduction of flavin (Roessler *et al*., 2009) (Fig. 2B). Mature poly-iron sulphur cluster proteins can be exported by the TAT translocase into the periplasmic compartment (Gralnick *et al*., 2006). It is therefore conceivable that proteins bearing such clusters could also be 'captured' by large outer-membrane β-barrel proteins.

Conclusion

Characterization of the MtrF structure has provided insights into the function of a key family of extracellular electron transfer proteins in *Shewanella* that reveal a remarkable evolutionary response to the natural complexities of Fe(III) and Mn(III/IV) minerals in the environment. Typically, these oxides and phyllosilicates are dispersed and small in size, exhibit complex morphologies and surface structures, and are often of limited physical accessibility because of residence in particle aggregates and coatings, and grain interiors. The MtrF structure is conducive to multi-modal electron transfer through direct contact with the surface of polyvalent metal oxides, and engagement with water-soluble flavins that may, in their reduced forms, distribute electron density to the surfaces of physically inaccessible oxide forms or other extracellular cytochromes. This versatility allows for respiration and electron disposal in different geochemical environments where the oxidized forms of polyvalent metals may exist in highly varied states as a result of lithology, drainage, sediment/soil age and weathering/biogeochemical processes. Furthermore, the MtrAB porin–cytochrome complex that enables electron transfer across the outer membrane in *Shewanella* may be a common motif that allows phylogenetically diverse organisms to engage in electron exchange with their surrounding environment. The stage is now set for structural, biochemical, microbiological and environmental analyses to test the models presented here and refine our appreciation of microbe-to-mineral electron transfer at the molecular level.

Acknowledgements

D. J. R. is a Royal Society Wolfson Foundation Merit Award holder. T. A. C. is a Research Council UK Fellow. The research from the author's laboratories was supported by the Biotechnology and Biological Sciences Research Council (H007288/1) and the EMSL Scientific Grand Challenge project at the W. R. Wiley Environmental Molecular Sciences Laboratory, a national scientific user facility sponsored by the US Department of Energy, Office of Biological and Environmental Research program located at Pacific Northwest National Laboratory. The Pacific Northwest National Laboratory is operated for the Department of Energy by Battelle.

References

Afkar, E., Reguera, G., Schiffer, M., and Lovley, D.R. (2005) A novel *Geobacteraceae*-specific outer membrane protein J (OmpJ) is essential for electron transport to Fe(III) and Mn(IV) oxides in *Geobacter sulfurreducens*. *BMC Microbiol* **5:** 41.

Baron, D., LaBelle, E., Coursolle, D., Gralnick, J.A., and Bond, D.R. (2009) Electrochemical measurement of electron transfer kinetics by *Shewanella oneidensis* MR-1. *J Biol Chem* **284:** 28865–28873.

Bouhenni, R.A., Vora, G.J., Biffinger, J.C., Shirodkar, S., Brockman, K., Ray, R., *et al*. (2010) The role of *Shewanella oneidensis* MR-1 outer surface structures in extracellular electron transfer. *Electroanalysis* **22:** 856–864.

Burns, J.L., Ginn, B.R., Bates, D.J., Dublin, S.N., Taylor, J.V., Apkarian, R., *et al*. (2010) Outer membrane-associated serine protease involved in adhesion of *Shewanella oneidensis* to Fe(III) oxides. *Environ Sci Technol* **44:** 68–73.

von Canstein, H., Ogawa, J., Shimizu, S., and Lloyd, J.R. (2008) Secretion of flavins by *Shewanella* species and their role in extracellular electron transfer. *Appl Environ Microbiol* **74:** 615–623.

Cao, B., Shi, L., Brown, R.N., Xiong, Y., Fredrickson, J.K., Romine, M.F., *et al*. (2011) Extracellular polymeric substances from *Shewanella* sp. HRCR-1 biofilms: characterization by infrared spectroscopy and proteomics. *Environ Microbiol* **13:** 1018–1031.

Clarke, T.A., Cole, J.A., Richardson, D.J., and Hemmings, A.M. (2007) The crystal structure of the pentahaem c-type cytochrome NrfB and characterization of its solution-state interaction with the pentahaem nitrite reductase NrfA. *Biochem J* **406:** 19–30.

Clarke, T.A., Holley, T., Hartshorne, R.S., Fredrickson, J.K., Zachara, J.M., Shi, L., and Richardson, D.J. (2008) The role of multihaem cytochromes in the respiration of nitrite in *Escherichia coli* and Fe(III) in *Shewanella oneidensis*. *Biochem Soc Trans* **36:** 1005–1010.

Clarke, T.A., Edwards, M.J., Gates, A.J., Hall, A., White, G.F., Bradley, J., *et al*. (2011) Structure of a bacterial cell surface decaheme electron conduit. *Proc Natl Acad Sci USA* **108:** 9284–9389.

Coursolle, D., Baron, D.B., Bond, D.R., and Gralnick, J.A. (2010) The Mtr respiratory pathway is essential for reducing flavins and electrodes in *Shewanella oneidensis*. *J Bacteriol* **192:** 467–474.

DiChristina, T.J., Moore, C.M., and Haller, C.A. (2002) Dissimilatory Fe(III) and Mn(IV) reduction by *Shewanella putrefaciens* requires *ferE*, a homolog of the *pulE* (*gspE*) type II protein secretion gene. *J Bacteriol* **184:** 142–151.

Dohnalkova, A.C., Marshall, M.J., Arey, B.W., Williams, K.H., Buck, E.C., and Fredrickson, J.K. (2011) Imaging hydrated microbial extracellular polymers: comparative analysis by electron microscopy. *Appl Environ Microbiol* **77:** 1254–1262.

Donald, J.W., Hicks, M.G., Richardson, D.J., and Palmer, T. (2008) The c-type cytochrome OmcA localizes to the outer membrane upon heterologous expression in *Escherichia coli*. *J Bacteriol* **190:** 5127–5131.

Eggleston, C.M., Vöro, J., Shi, L., Lower, B.H., Droubay, T.C., and Colberg, P.J.S. (2008) Binding and direct electrochemistry of OmcA, an outer-membrane cytochrome from an iron reducing bacterium, with oxide electrodes: a candidate biofuel cell system. *Inorganica Chim Acta* **361:** 769–777.

El-Naggar, M.Y., Wanger, G., Leung, K.M., Yuzvinsky, T.D., Southam, G., Yang, J., *et al*. (2010) Electrical transport along bacterial nanowires from *Shewanella oneidensis* MR-1. *Proc Natl Acad Sci USA* **107:** 18127–18131.

Firer-Sherwood, M., Pulcu, G.S., and Elliott, S.J. (2008) Electrochemical interrogations of the Mtr cytochromes from *Shewanella*: opening a potential window. *J Biol Inorg Chem* **13:** 849–854.

Firer-Sherwood, M.A., Ando, N., Drennan, C.L., and Elliott, S.J. (2011) Solution-based structural analysis of the decaheme cytochrome, MtrA, by small-angle X-ray scattering and analytical ultracentrifugation. *J Phys Chem B* **115:** 11208–11214.

Gorby, Y.A., Yanina, S., McLean, J.S., Rosso, K.M., Moyles, D., Dohnalkova, A., *et al*. (2006) Electrically conductive bacterial nanowires produced by *Shewanella oneidensis* strain MR-1 and other microorganisms. *Proc Natl Acad Sci USA* **103:** 11358–11363.

Gralnick, J.A., Vali, H., Lies, D., and Newman, D.K. (2006) Extracellular respiration of dimethyl sulfoxide by *Shewanella oneidensis* strain MR-1. *Proc Natl Acad Sci USA* **103:** 4669–4674.

Hartshorne, R.S., Jepson, B.N., Clarke, T.A., Field, S.J., Fredrickson, J., Zachara, J., *et al*. (2007) Characterization of *Shewanella oneidensis* MtrC: a cell-surface decaheme cytochrome involved in respiratory electron transport to extracellular electron acceptors. *J Biol Inorg Chem* **12:** 1083–1094.

Hartshorne, R.S., Reardon, C.L., Ross, D., Nuester, J., Clarke, T.A., Gates, A.J., *et al*. (2009) Characterization of an electron conduit between bacteria and the extracellular environment. *Proc Natl Acad Sci USA* **106:** 22169–22174.

Heidelberg, J.F., Paulsen, I.T., Nelson, K.E., Gaidos, E.J., Nelson, W.C., Read, T.D., *et al*. (2002) Genome sequence of the dissimilatory metal ion-reducing bacterium *Shewanella oneidensis*. *Nat Biotechnol* **20:** 1118–1123.

Jensen, H.M., Albers, A.E., Malley, K.R., Londer, Y.Y., Cohen, B.E., Helms, B.A., *et al*. (2010) Engineering of a synthetic electron conduit in living cells. *Proc Natl Acad Sci USA* **107:** 19213–19218.

Jiang, X., Hu, J., Fitzgerald, L.A., Biffinger, J.C., Xie, P., Ringeisen, B.R., and Lieber, C.M. (2010) Probing electron transfer mechanisms in *Shewanella oneidensis* MR-1 using a nanoelectrode platform and single-cell imaging. *Proc Natl Acad Sci USA* **107:** 16806–16810.

Jiao, Y., and Newman, D.K. (2007) The *pio* operon is essential for phototrophic Fe(II) oxidation in *Rhodopseudomonas palustris* TIE-1. *J Bacteriol* **189:** 1765–1773.

Johs, A., Shi, L., Droubay, T., Ankner, J.F., and Liang, L. (2010) Characterization of the decaheme c-type cytochrome OmcA in solution and on hematite surfaces by small angle X-ray scattering and neutron reflectometry. *Biophys J* **98:** 3035–3043.

Kerisit, S.N., Rosso, K.M., Dupuis, M., and Valiev, M. (2007) Molecular computational investigation of electron transfer kinetics across cytochrome-iron oxide interfaces. *J Phys Chem C* **111:** 11363–11375.

Leang, C., Qian, X., Mester, T., and Lovley, D.R. (2010) Alignment of the c-type cytochrome OmcS along pili of *Geobacter sulfurreducens*. *Appl Environ Microbiol* **76:** 4080–4084.

Lies, D.P., Hernandez, M.E., Kappler, A., Mielke, R.E., Gralnick, J.A., and Newman, D.K. (2005) *Shewanella oneidensis* MR-1 uses overlapping pathways for iron reduction at a distance and by direct contact under conditions relevant for Biofilms. *Appl Environ Microbiol* **71:** 4414–4426.

Liu, J., Wang, Z., Belchik, S.M., Edwards, M.J., Liu, C., Kennedy, D.W., *et al*. (2012) Identification and characterization of MtoA: a decaheme c-type cytochrome of the neutrophilic Fe(II)-oxidizing bacterium *Sideroxydans lithotrophicus* ES-1. *Front Microbiol* **3:** 37.

Lower, B.H., Shi, L., Yongsunthon, R., Droubay, T.C., McCready, D.E., and Lower, S.K. (2007) Specific bonds between an iron oxide surface and outer membrane cytochromes MtrC and OmcA from *Shewanella oneidensis* MR-1. *J Bacteriol* **189:** 4944–4952.

Lower, B.H., Lins, R.D., Oestreicher, Z., Straatsma, T.P., Hochella, M.F., Jr, Shi, L., and Lower, S.K. (2008) In vitro evolution of a peptide with a hematite binding motif that may constitute a natural metal-oxide binding archetype. *Environ Sci Technol* **42:** 3821–3827.

Lower, B.H., Yongsunthon, R., Shi, L., Wildling, L., Gruber, H.J., Wigginton, N.S., *et al*. (2009) Antibody recognition force microscopy shows that outer membrane cytochromes OmcA and MtrC are expressed on the exterior surface of *Shewanella oneidensis* MR-1. *Appl Environ Microbiol* **75:** 2931–2935.

McLean, J.S., Pinchuk, G.E., Geydebrekht, O.V., Bilskis, C.L., Zakrajsek, B.A., Hill, E.A., *et al*. (2008) Oxygen-dependent autoaggregation in *Shewanella oneidensis* MR-1. *Environ Microbiol* **10:** 1861–1876.

Malvankar, N.S., Vargas, M., Nevin, K.P., Franks, A.E., Leang, C., Kim, B.C., *et al*. (2011) Tunable metallic-like conductivity in microbial nanowire networks. *Nat Nanotechnol* **6:** 573–579.

Malvankar, N.S., Tuominen, M.T., and Lovley, D.R. (2012) Comment on 'On electrical conductivity of microbial nanowires and biofilms' by S. M. Strycharz-Glaven, R. M. Snider, A. Guiseppi-Elie and L. M. Tender, Energy Environ. Sci., 2011, 4, 4366. *Energy Environ Sci* **5:** 6247–6249.

Marsili, E., Baron, D.B., Shikhare, I.D., Coursolle, D., Gralnick, J.A., and Bond, D.R. (2008) *Shewanella* secretes flavins that mediate extracellular electron transfer. *Proc Natl Acad Sci USA* **105:** 3968–3973.

Meitl, L.A., Eggleston, C.M., Colberg, P.J.S., Khare, N., Reardon, C.L., and Shi, L. (2009) Electrochemical interaction of *Shewanella oneidensis* MR-1 and its outer membrane cytochromes OmcA and MtrC with hematite electrodes. *Geochim Cosmochim Acta* **73:** 5292–5307.

Myers, J.M., and Myers, C.R. (2000) Role of the tetraheme cytochrome CymA in anaerobic electron transport in cells of *Shewanella putrefaciens* MR-1 with normal levels of menaquinone. *J Bacteriol* **182:** 67–75.

Peretyazhko, T., Zachara, J.M., Kennedy, D.W., Fredrickson, J.K., Arey, B.W., McKinley, J.P., *et al*. (2010) Ferrous phosphate surface precipitates resulting from the reduction of intragrain 6-line ferrihydrite by *Shewanella oneidensis* MR-1. *Geochim Cosmochim Acta* **74:** 3751–3767.

Pessanha, M., Rothery, E.L., Miles, C.S., Reid, G.A., Chapman, S.K., Louro, R.O., *et al*. (2009) Tuning of functional heme reduction potentials in *Shewanella* fumarate reductases. *Biochim Biophys Acta* **1787:** 113–120.

Qian, X., Mester, T., Morgado, L., Arakawa, T., Sharma, M.L., Inoue, K., *et al*. (2010) Biochemical characterization of purified OmcS, a c-type cytochrome required for insoluble Fe(III) reduction in *Geobacter sulfurreducens*. *Biochim Biophys Acta* **1807:** 404–412.

Reardon, C.L., Dohnalkova, A.C., Nachimuthu, P., Kennedy,

D.W., Saffarini, D.A., Arey, B.W., *et al*. (2010) Role of outer-membrane cytochromes MtrC and OmcA in the biomineralization of ferrihydrite by *Shewanella oneidensis* MR-1. *Geobiology* **8:** 56–68.

Reguera, G., McCarthy, K.D., Mehta, T., Nicoll, J.S., Tuominen, M.T., and Lovley, D.R. (2005) Extracellular electron transfer via microbial nanowires. *Nature* **435:** 1098–1101.

Reguera, G., Nevin, K., Nicoll, J.S., Covalla, S.F., Woodard, T.L., and Lovley, D.R. (2006) Biofilm and nanowire production leads to increased current in *Geobacter sulfurreducens* fuel cells. *Appl Environ Microbiol* **72:** 7345–7348.

Roessler, M.M., King, M.S., Robinson, A.J., Armstrong, F.A., Harmer, J., and Hirst, J. (2009) Direct assignment of EPR spectra to structurally defined iron-sulfur clusters in complex I by double electron-electron resonance. *Proc Natl Acad Sci USA* **107:** 1930–1935.

Ross, D.E., Ruebush, S.S., Brantley, S., Hartshorne, R.S., Clarke, T.A., Richardson, D.J., and Tien, M. (2007) Characterization of protein-protein interactions involved in iron reduction by *Shewanella oneidensis* MR-1. *Appl Environ Microbiol* **73:** 5797–5808.

Schuetz, B., Schicklberger, M., Kuermann, J., Spormann, A.M., and Gescher, J. (2009) Periplasmic electron transfer via the c-type cytochromes MtrA and FccA of *Shewanella oneidensis* MR-1. *Appl Environ Microbiol* **75:** 7789–7796.

Shi, L., Chen, B., Wang, Z., Elias, D.A., Mayer, M.U., Gorby, Y.A., *et al*. (2006) Isolation of a high-affinity functional protein complex between OmcA and MtrC: two outer membrane decaheme *c*-type cytochromes of *Shewanella oneidensis* MR-1. *J Bacteriol* **188:** 4705–4714.

Shi, L., Deng, S., Marshall, M.J., Wang, Z., Kennedy, D.W., Dohnalkova, A.C., *et al*. (2008) Direct involvement of type II secretion system in extracellular translocation of *Shewanella oneidensis* outer membrane cytochromes MtrC and OmcA. *J Bacteriol* **190:** 5512–5516.

Smith, D.M., Rosso, K.M., Dupuis, M., Valiev, M., and Straatsma, T. (2006) Electronic coupling between heme electron-transfer centers and its decay with distance depends strongly on relative orientation. *J Phys Chem B* **110:** 15582–15588.

Strycharz-Glaven, S.M., and Tender, L.M. (2012) Reply to the 'Comment on "On electrical conductivity of microbial nanowires and biofilms" ' by N. S. Malvankar, M. T. Tuominen and D. R. Lovley, *Energy Environ. Sci*., 2012, 5, DOI: 10.1039/c2ee02613a. *Energy Environ Sci* **5:** 6250–6255.

Strycharz-Glaven, S.M., Snider, R.M., Guiseppe-Elie, A., and Tender, L.M. (2011) On electrical conductivity of microbial nanowires and biofilms. *Energy Environ Sci* **4:** 4366–44379.

Taylor, P., Pealing, S.L., Reid, G.A., Chapman, S.K., and Walkinshaw, M.D. (1999) Structural and mechanistic mapping of a unique fumarate reductase. *Nat Struct Biol* **6:** 1108–1112.

Xiong, Y., Shi, L., Chen, B., Mayer, M.U., Lower, B.H., Londer, Y., *et al*. (2006) High-affinity binding and direct electron transfer to solid metals by the *Shewanella oneidensis* MR-1 outer membrane *c*-type cytochrome OmcA. *J Am Chem Soc* **128:** 13978–13979.

Zargar, K., and Saltikov, C.W. (2009) Lysine-91 of the tetraheme c-type cytochrome CymA is essential for quinone interaction and arsenate respiration in *Shewanella* sp. strain ANA-3. *Arch Microbiol* **191:** 797–806.

Zhang, H., Tang, X., Munske, G.R., Zakharova, N., Yang, L., Zheng, C., *et al*. (2008) In vivo identification of the outer membrane protein OmcA-MtrC interaction network in *Shewanella oneidensis* MR-1 cells using novel hydrophobic chemical cross-linkers. *J Proteome Res* **7:** 1712–1720.

Zhang, H., Tang, X., Munske, G.R., Tolic, N., Anderson, G.A., and Bruce, J.E. (2009) Identification of protein-protein interactions and topologies in living cells with chemical cross-linking and mass spectrometry. *Mol Cell Proteomics* **8:** 409–420.